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静岡大学地球科学研究報告 28 (2001年7月)75頁～82頁
Geosci. Repts. Shizuoka Univ., 28 (July, 2001), 75-82

Isolation of nano-colloidal organic matter from coastal Bora Bay seawater using porous glass filter as cross-flow filtration membrane

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Abstract A new type inorganic membrane-porous glass is applied for both cross-flow filtration modes. The laboratory results show that permeation of Albumin passing through 30 nm porous glass filter can be negligible (<0.015%). The filtration behavior of natural DOC consistently shows a decreasing concentration in the permeate with increasing CFs. Thus, the permeation of HMW molecules is minimal during CFF using porous glass filter, even under high CFs ($CF>50$). During filtration of Bora Bay seawater, OC concentration of permeate (C_p) first is at very beginning (*i.e.* in the first liter of permeate) higher than the OC concentration of pre-filter sample, and then lower and lower with permeate volume and concentration factor increasing. The speed of OC concentration decreasing is very rapid at beginning. After the $CF>4$ using regular concentration mode and after CF>12 using diafiltration mode, the variation of C_p becomes slightly as CF continuously increased. Thus, the organic matter of Bora Bay seawater can easily pass through 30 nm porous glass filter, in another word, a large amount of organic matters of Bora Bay seawater can not be retained by 30 nm porous glass filter. The results show that major organic matters of Bora Bay seawater are smaller than 30 KDa.

Key Words: colloids; organic matter; porous glass filter; cross-flow filtration

Introduction

The previous studies have shown that sub-μm sized colloids are abundant in seawater and the colloidal phase can comprise a significant fraction of dissolved organic matter in seawater. Some fractions of marine DOM may have high turnover rates, and processes which influence marine DOM cycling may have significant ramifications to global carbon studies. Identifying and characterizing the marine colloidal phase is important for understanding the biogeochemical cycling of organic carbon and nutrients.

The cross flow filtration (CFF) has been most extensively used as an isolation tool to study colloids in seawater for understanding the cycling of both inorganic and organic elements and materials (Bauer *et al.*, 1996; Buesseler, 1996; Guo, 1996; Powell, 1996; Wen, 1996). Until now, almost all studies have been done using the organic membrane for separating organic colloidal matters from seawater. Here, it is reported for the first time to apply a new kind of inorganic membrane (porous glass filter) for isolation organic colloids from seawater.

In a few previous studies where highly concentrated colloidal organic matter was analyzed for organic nitrogen content (Benner, 1992; Sigleo, 1983; Pakulski & Benner, 1992) highly C/N ratios of 20-25 have generally been observed. Bauer (1996) studied the ratios of C/N in different fractions, the elevated C/N ratios of the HMW fraction were also observed in several open-ocean samples which colloidal organic fraction is a nitrogen-depleted (C/N?20-22) and LMW organic fraction is a highly nitrogen-enriched (C/N?2-5).

We measured carbon, nitrogen and phosphorus contents of: (1) particulate carbon and nitrogen; (2) total starting DOM prior to processing by CFF; (3) HMW or colloidal organic matter retained by porous glass filter; and (4) LMW organic matter that comprises the permeate or filtrate.

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Table 1 Sampling ancillary data.

Station	Sampling Depth (m)	Salinity (‰)	Temperature (℃)	PC (μM)	PC/PN
M1	2.8	33.05	31	5.09	10.06
M3	2.8	34.23	29	4.63	10.05
L1	4.3	34.21	31	5.18	9.37
L2	3.6	33.88	30	6.64	10.51

Methods

Sample collection

Samples were taken at 4 stations located in Bora Bay of Miyagojima of Okinawa during high tide on 30 July, 2000. The sampling ancillary data are listed in Table 1.

All samples were filtered through precombusted (6 h at 480℃) Advantec GF-75 glass-fiber filters (0.3 μm, 90mm diameter) and collected into polycarbonate bottles and kept in low temperature till to be separated by CFF and analyzed.

Porous glass filter

The porous glass filter (abbreviation as PG) which is made of borosilicate glass is a new kind of inorganic filter. Its pore size is 30 nm and porosity is move than 50%. The contour of PG filter is tube or disk. In our previous studies, the results showed that the porous glass filter is suitable to be used in pH<9.0. The amount of residue carbon in PG filter is lower than the detection limit while PG is combusted over 2 hours at 600℃. It is suggested that the porous glass filter is suitable to isolate organic colloidal materials from natural water.

CFF system

The porous glass filter was used in two configurations CFF system (Fig.1). One is a regular concentration mode which the pre-filter sample is concentrated from a single sample reservoir. In this case, the concentration factor, *CF* can be calculated from

$$CF = \text{(initial sample volume)}/\text{(final retentate volume)}. \quad (1)$$

Another is diafiltration mode which the retentate reservoir is considerably smaller than the sample volume and the retentate volume was maintained constant by continuously supplied with fresh sample for replacing the permeate volume removed. In this case,

$$CF = [\text{(permeate volume)} + \text{(retentate volume)}]/\text{(retentate volume)} \quad (2)$$

In CFF, colloidal abundance are defined by their retention relative to a given CFF membrane, as calculated from

$$C_{coll} = (C_r - C_p)/CF, \quad (3)$$

where C_r and C_p are the concentration in the retentate and permeate, respectively.

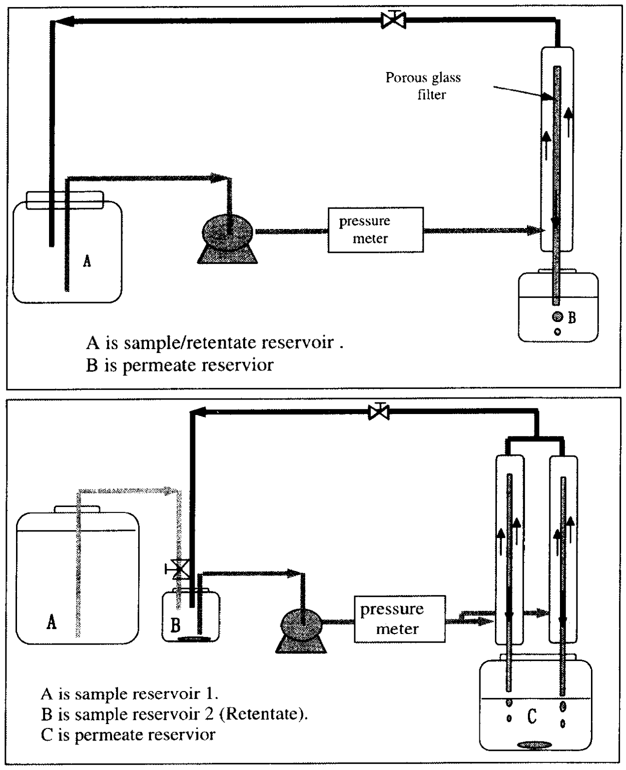

Fig. 1 Schematic of the cross-flow filtration systems used in this study. All joins, tubings and valves are made of Teflon.

When used in the regular concentration mode, the reservoir volume decreases from an initial sample volume of 10 ℓ to a final retentate volume of 0.5-l (*i. e. CF*=20). In the diafiltration mode, the retentate volume is maintained constant in a 0.5-l container. The recycling flow rate is 30 mℓ/min with a trans-porous glass flow rate of 10 mℓ/min, and the trans-porous glass filter pressure, which is controlled by valve on the retentate line and by the pump speed, is 5 kg/cm^2.

All filtrations are carried out in a Class-100 laminar flow clean bench.

Cleaning process

Before porous glass filter was used, it was combusted at 600℃ for 3 h. The CFF system was extensively cleaned before processing each sample with 4 mol/ℓ HCl under pressure (5 Kg/cm^2) by recycling for one hour and allowed to stand for at least half

Table 2 Particulate carbon and nitrogen concentrations.

Sample ID	PC (μ M)	PN (μ M)	PC/PN
L1	5.179	0.5507	9.37
L2	6.639	0.6326	10.51
M1	5.090	0.5027	10.06
M3	4.628	0.4611	10.05

hour. After the acid solution cleaning, the system was flushed with Milli-Q water until the pH was the same as in the Milli-Q water. These initial cleaning steps were followed by pre-filtrated sample for condition the porous glass prior to the preconditioning run. During the preconditioning, the sample was allowed to run through the permeate line, and then both permeate and retentate solutions dwascarded. The preconditioning was both to reduce contamination of extracterbale from system, and potentially to minimize sorptive losses to the membrane and other surfaces (Buesseler, 1996). Following the preconditoning, the CFF system was completely drained and was flushed with 1-l sample solution, and CFF processing began immediately.

Analytical procedures

All samples (pre-filtered samples, permeates and retentates) that were collected and analyzed for total organic carbon, total nitrogen, total phosphorus, nitrite and nitrate, ammonium and phosphate. Particulate carbons and nitrogens were measured by high temperature combustion method using a Sumigraph NC-90 A. Dissolved Organic carbons were assayed by high temperature catalytic oxidation (HTCO) using a Shimadzu TOC-5000 analyzer. Total nitrogen and total phosphorus were analyzed using a persulfate oxidation method. $NO_3^- + NO_2^-$, NO_2^-, NH_4^+ and PO_4^{3-} were all determined by continue flow analysis using a TRAACS 2000 autoanalyzer (Bran • Luebbe, Japan).

Results and discussions

Particulate carbon and nitrogen

The particulate carbon and nitrogen (>0.3 μm) concentrations are showed in Table 2. The concentrations of particulate carbon and nitrogen are very low. Concentrations of particulate carbon and nitrogen in each station seawater are slight difference, but the ratios of PC/PN are very similar, about 10.

Retention behavior

At any point in the filtration process, the retention coefficient (%*R*) is defined as the percentage reduction in the feed concentration (C_f) across the membrane (*e. g.*, Kilduff & Weber, 1992):

$$\%R = (1 - C_p/C_f) \times 100\%, \quad (4)$$

where C_p is the OC concentration in the integral permeate and C_f is the DOC concentration in the feed. The Fig. 2 shows the permeate concentrations during time-series experiments using natural seawater from Bora Bay vary with permeate volume and concentration factor increasing. The results are clear that retention coefficients of porous glass filter increase with *CF* increasing. The final average retention coefficients of Bora Bay seawater using diafiltration and regular concentration mode are 7.97% (final *CF*=13.08) and 7.68% (final *CF*=16.91), respectively.

Our experimental data during retention experiment showed completely contrast trend comparing with many previous results using organic membranes (Guo, 1996; 2000; Wen, 1996). As Guo (1996) pointed that it was clear that apparent values of colloidal fractions decreased with increasing concentration factors. Guo (2000) pointed that the ultrafiltration behavior of natural DOC and LMW metals could be well predicted by a permeation model, and consistently showed an increasing concentration in the permeate with increasing *CF*.

However, the results of mass balances using natural seawater and Albumin solution showed very difference in porous glass CFF system. Mass balance of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) during filtration natural seawater were in the range of 92.91 %~105.9 % and 91.6 %~130.6 % and 85.5~138.0 %, respectively. But it is just 12.09 % (*CF*=11.28) or 2.27% (*CF*=63.37) of DOC using diafiltration mode or concentration mode. It shows the absorption of Albumin in surface of porous glass is main problem. The higher the *CF* is, the larger the loss amount of Albumin is.

Breakthrough

In any CFF phase speciation study, it is crucial to identify an optimal concentration factor that will not lead to serious alternations of DOC concentrations in permeate and retentate phase. A low concentration factor may lead to incomplete separation, while high concentration factors may lead to breakthrough due to 3-D steric and concentration polarization effects.

Time series samples from permeate line were collected as discrete samples using natural seawater as well as macromolecular solutions (*S*=30 ‰) to

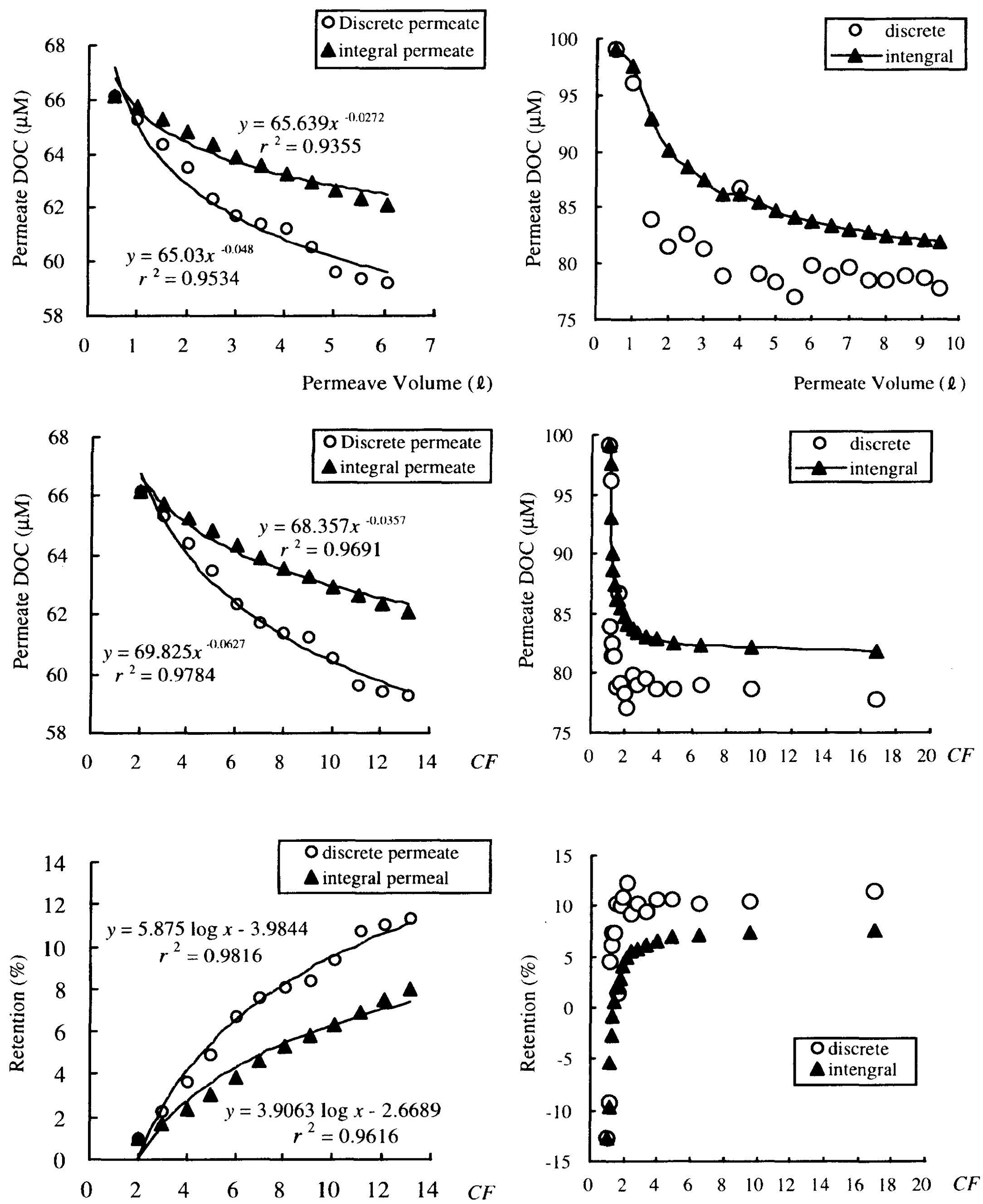

Fig. 2 Variations of the concentration of organic carbon in discrete permeate samples and the retention percentage with permeate volume and concentration factors increasing using natural seawater from Bora Bay.

determine the appropriate concentration factor and to examine how filtration behavior of natural DOC is using 30 nm porous glass filter or how macromolecular DOM is being retained by 30 nm porous glass filter. Duplicate experiments were carried out for both CFF modes. The final retentate was also collected. Tables 3 and 4 and Fig. 3 showed how concentration of OC in permeate varied with permeate volume and concentration factor (*CF*) increasing.

As can be seen in Table 3, permeation of Albumin passing through 30 nm porous glass filter can be negligible during both diafiltration and regular concentration. Thus, the permeation of HMW molecules is minimal during CFF with porous glass filter, even under high *CF* (*CF*>50).

The filtration behavior of natural DOC consistently showed a decreasing concentration in the permeate with increasing *CF*. During filtration of Bora Bay seawater, OC concentration of permeate (C_p) first is at very beginning (*i. e.* in the first liter of permeate) higher than the OC concentration of pre-filter sample, and then lower and lower with permeate volume and concentration factor

Table 3 Results of filtration breakthrough of Albumin (NMW 66,000) while *CF* increasing.

Albumin 30‰ NaCl	sample ID	*CF*	Volume (mℓ)	fliw rate (mℓ/min)	DOC (μM C)
diafiltration CFF	sample		2255.1		156.54
	retentate		200.0		199.03
	Permeate1#	2.03	206.0	8.41	2.02
	Permeate2#	3.04	202.7	8.34	2.01
	Permeate3#	4.05	201.2	8.47	0.45
	Permeate4#	5.04	198.2	8.69	1.18
	Permeate5#	6.06	204.4	8.73	1.55
	Permeate6#	7.07	201.1	8.79	2.07
	Permeate7#	8.12	211.2	8.85	3.09
	Permeate8#	9.13	201.7	8.89	1.74
	Permeate9#	10.28	228.8	9.09	-0.69
	Permeate10#	11.28	199.9	0.00	-0.71
	Mass balance				12.09%
concentration CFF	sample	0.00	6267.5	0.00	156.54
	retentate	63.37	98.9	0.00	225.63
	Permeate1#	1.06	379.4	8.43	-1.31
	Permeate2#	1.15	435.4	8.32	-3.95
	Permeate3#	1.25	439.0	8.25	-0.87
	Permeate4#	1.38	459.5	8.16	0.53
	Permeate5#	1.54	476.7	8.14	-0.72
	Permeate6#	1.75	493.3	8.18	-8.40
	Permeate7#	2.00	451.5	8.29	-2.25
	Permeate8#	2.18	253.5	8.28	-4.58
	Permeate9#	2.59	455.1	8.25	-5.98
	Permeate10#	3.18	455.9	8.22	-5.36
	Permeate11#	4.14	453.0	8.22	-1.07
	Permeate12#	5.89	450.8	8.14	-5.03
	Permeate13#	10.26	453.7	8.21	-2.04
	Permeate14#	15.07	194.8	8.18	-2.44
	Permeate15#	29.12	200.6	8.09	-1.01
	Permeate16#	63.37	116.3	8.06	0.89
	Mass balance				2.27%

increasing. The speed of OC concentration decreasing was very rapid at beginning. After the *CF*>4 using regular concentration mode and after *CF*>12 using diafiltration mode, the variation of C_p became slightly as *CF* continual increased. Thus, the organic matter of Bora Bay seawater can easily pass through 30 nm porous glass filter, in another word, a large amount of organic matters of Bora Bay seawater can not be retained by 30 nm porous glass filer.

Results of Bora Bay seawater

Results of DOC (Fig. 3) and autoanalyzer TDN, TDP, $NO_3^-+NO_2^-$, NO_2^--, NH_4^+ and PO_4^+ concentrations in pre-filtered samples and in CFF processed permeates and retentates using diafiltration mode are shown in Table 5. Permeate samples were collected at a concentration factor of two (*CF*=2) and again when the sample had been concentrated to its final volume. Retentate samples were collected at *CF*= final only.

In almost all samples at Bora Bay when the abundant of colloidal OC was low, OC concentration in permeate decreased between *CF*=2 and *CF* =final, and the OC concentrations in permeate at *CF*=2 were all higher than in pre-filtered sample.

Table 4 Bora Bay seawater data in time series experiments.

Sample ID diafiltration mode	*CF*	Volume (mℓ)	DOC μM	DON μM	DOP μM	DIN μM	PO_4^{3-} μM
M1permeate1#	2.01	506.0	66.17	4.683	0.066	1.075	0.065
M1permeate2#	3.02	501.5	65.30	4.251	0.067	1.002	0.066
M1permeate3#	4.02	504.8	64.39	5.228	0.048	1.049	0.073
M1permeate4#	5.03	503.1	63.50	4.618	0.065	1.149	0.062
M1permeate5#	6.04	502.2	62.36	4.497	0.093	1.087	0.063
M1permeate6#	7.04	503.2	61.74	5.042	0.088	0.720	0.062
M1permeate7#	8.05	502.2	61.40	4.261	0.051	0.880	0.059
M1permeate8#	9.06	507.5	61.23	4.719	0.033	0.871	0.069
M1permeate9#	10.07	503.3	60.55	4.467	0.066	1.056	0.057
M1permeate10#	11.07	502.7	59.62	4.710	0.063	0.849	0.057
M1permeate11#	12.09	506.0	59.41	4.998	0.081	0.860	0.055
M1permeate12#	13.08	499.0	59.27	4.546	0.054	0.900	0.062
M1Retentate	13.08	488.3	65.64	3.744	0.048	0.866	0.055
M1prefiltered water(<0.3um)		-	66.82	4.128	0.094	0.866	0.038
mass balance			92.91%			110.1%	162.2%

Sample ID concentration mode	*CF*	Volume (mℓ)	DOC μM	DON μM	DOP μM	DIN μM	PO43- μM
L1permeate1#	1.043	500.7	99.12	4.487	0.077	0.886	0.045
L1permeate2#	1.101	499.8	96.08	4.302	0.079	0.963	0.049
L1permeate3#	1.165	506.0	83.90	4.670	0.103	0.865	0.031
L1permeate4#	1.238	502.8	81.45	4.950	0.069	0.867	0.035
L1permeate5#	1.32	500.0	82.56	4.304	0.080	0.948	0.053
L1permeate6#	1.413	502.6	81.43	4.405	0.077	0.903	0.046
L1permeate7#	1.521	502.4	78.91	4.705	0.067	0.827	0.060
L1permeate8#	1.648	503.3	86.74	4.585	0.074	0.849	0.039
L1permeate9#	1.795	497.7	79.12	4.106	0.083	0.873	0.049
L1permeate10#	1.971	499.4	78.29	4.780	0.065	0.935	0.049
L1permeate11#	2.189	504.8	77.09	4.609	0.086	0.855	0.039
L1permeate12#	2.459	500.3	79.85	4.846	0.074	0.916	0.055
L1permeate13#	2.806	503.5	78.97	4.651	0.080	0.851	0.045
L1permeate14#	3.264	500.2	79.58	4.584	0.078	0.855	0.046
L1permeate15#	3.895	496.2	78.62	4.673	0.085	0.925	0.030
L1permeate16#	4.849	505.1	78.63	4.659	0.057	1.010	0.057
L1permeate17#	6.483	519.8	78.97	5.384	0.049	0.875	0.068
L1permeate18#	9.508	490.9	78.71	4.517	0.064	0.921	0.063
L1permeate19#	16.91	460.4	77.85	4.441	0.071	0.968	0.051
L1Retentate	16.91	591.3	91.91	4.355	0.106	0.923	0.036
prefiltered water(<0.3um)			87.94	3.989	0.101	0.914	0.014
COC			0.832				
mass balance (%)			93.80%			96.02%	300.8%

The ratios of DOC to DON are in the range from~11 to~23. In contrast, the ratios of N/P of DOM varied in a large range from~29 to~84.

Conclusions

The laboratory results using Albumin standard molecule solution and natural seawater from Bora Bay show that permeation of HMW passing through 30 nm porous glass filter can be negligible (<0.015%). Thus, the breakthrough of HMW molecules is minimal during CFF using porous glass filter, even under high *CF* (*CF*>50).

The filtration behavior of natural DOC consistently showed a decreasing concentration in the permeate with increasing *CF*. This result is completely contrast to use organic membrane during CFF process.

OC concentration of permeate (C_p) from Bora

Table 5 Analytical data for pre-filtered and CFF-processed seawater of Bora Bay (2000.7.30. high tide).

station	Type	*CF*	DOC µmol/ ℓ	NO_3^-+NO_2^- µmol/ ℓ	NO_2^- µmol/ ℓ	NH_4^+ µmol/ ℓ	PO_4^+ µmol/ ℓ	TDN µmol/ ℓ	TDP µmol/ ℓ	DOC/ DON	DON/ DOP
L1	sample		64.20	0.890	0.071	0.052	0.070	5.219	0.121	15.26	83.21
L1	Permeate	2	66.05	0.896	0.057	0.070	0.086	4.542	0.153	18.77	52.87
L1	permeate	20	63.89	0.904	0.106	0.050	0.069	4.954	0.141	16.41	53.97
L1	retentate	20	63.29	0.903	0.060	0.005	0.075	5.105	0.155	15.30	51.93
Mass balance			98.6%	101.4%			106.5%	97.7%	127.6%		
L2	sample		63.93	0.767	0.079	0.035	0.046	4.576	0.121	17.30	49.28
L2	Permeate	2	65.25	0.848	0.091	0.116	0.076	5.090	0.149	16.17	55.13
L2	permeate	20	60.65	0.791	0.053	0.027	0.070	4.914	0.129	15.00	68.42
L2	retentate	20	63.05	0.970	0.198	-0.031	0.038	5.129	0.133	15.80	42.29
Mass balance			98.4%	125.3%			86.2%	111.8%	109.3%		
M1	sample		55.88	0.806	0.083	-0.044	0.052	4.732	0.181	14.38	30.14
M1	Permeate	2	59.09	0.841	0.078	0.148	0.067	5.354	0.177	13.78	38.78
M1	permeate	20	57.50	0.836	0.076	-0.016	0.068	5.300	0.143	13.06	58.69
M1	retentate	20	59.25	0.862	0.087	0.050	0.049	6.228	0.155	11.33	49.36
Mass balance			105.9%	106.7%			96.9%	130.6%	85.5%		
M3	sample		67.20	0.751	0.119	0.047	0.052	5.018	0.124	18.99	42.03
M3	Permeate	2	71.61	0.841	0.115	0.421	0.087	5.465	0.161	22.93	31.81
M3	permeate	20	67.16	0.678	0.067	0.052	0.064	4.786	0.122	20.28	35.76
M3	retentate	20	68.29	0.718	0.099	0.199	0.053	4.585	0.173	21.55	35.22
Mass balance			101.5%	95.4%			103.1%	91.6%	138.0%		
M3	sample		73.06	0.782	0.088	-0.027	0.050	4.690	0.141	16.38	57.33
M3	Permeate	2	76.45	1.199	0.146	0.179	0.053	4.858	0.158	17.52	54.75
M3	permeate	40	73.50	0.725	0.080	0.121	0.063	4.550	0.165	16.83	68.38
M3	retentate	40	75.05	0.698	0.063	0.259	0.052	4.502	0.151	19.14	29.76
Mass balance			101.6%	95.6%			102.6%	91.5%	139.0%		

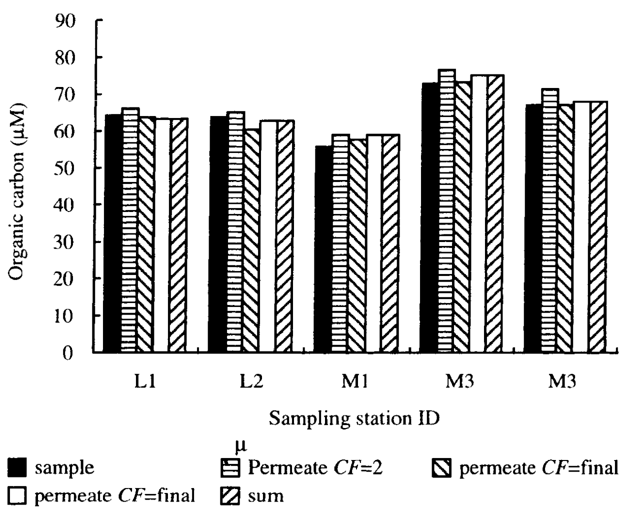

Fig. 3 The organic carbon distribution in all fractions of Bora Bay seawater.

Bay seawater first is at very beginning (*i. e.* in the first liter of permeate) higher than the OC concentration of pre-filter sample, but decreases rapidly with permeate volume and concentration factor increasing and becomes lower than the OC concentration of pre-filter sample. After the *CF*>4 using regular concentration mode and after *CF*>12 using diafiltration mode, the variation of C_p is slow as *CF* continual increased. Thus, the organic matter of Bora Bay seawater can easily pass through 30 nm porous glass filter. This results shows that major organic matters of Bora Bay seawater are smaller than 30 KDa.

Acknowledgements

This research work was supported, in parts, by RITE (Research Institute of Innovative Technology for the Earth) of Japan.

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